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Biophysical Chemistry 99 (2002) 43–53

Biophysical Chemistry

www.elsevier.com/locate/bpc

Proton concentration (pH) switches the higher-order structure of DNA in the presence of spermine

Naoko Makita[a,b], Kenichi Yoshikawa[b,*]

[a]*Graduate School of Human Informatics, Nagoya University, Nagoya 464-8601, Japan*
[b]*Department of Physics, Graduate School of Science, Kyoto University and CREST, Kyoto, 606-8502, Japan*

Received 13 February 2002; received in revised form 11 April 2002; accepted 12 April 2002

Abstract

Single-chain observations on the conformational change of giant DNA (T4 DNA) molecules were performed using fluorescence microscopy at different values of pH in the presence of spermine. Individual DNA molecules undergo a large discrete change, or all-or-none transition, in conformation from a folded compact state to an unfolded coil state with an increase in pH. This abrupt unfolding of DNA with an increase in pH is attributed to a decrease in the concentration of the tetravalent form in spermine [SPM^{4+}]. We propose a scheme for the folding transition of single DNAs, where the manner of spermine binding changes dramatically from weak loose binding in the elongated coil state to strong tight binding in the folded compact state. We discuss the hierarchical nature of the transition, i.e. cooperative continuous change on the ensemble vs. all-or-none switching on individual DNAs.

Keywords: Single molecular-chain observation; Higher-order structure of DNA; Folding/unfolding transition; Switching of conformation; Polyamine; Non-specific interaction

1. Introduction

The change in the morphology of DNA molecules between a compact state and a loosened state is a common feature of native genomes. Viral genomes are compacted by internal viral proteins and polyamines, and are unfolded for gene expression after infection of cells [1,2]. In both prokaryotes and eukaryotes, giant DNA molecules of mm to cm in length are packed in a narrow space on the order of micrometers. The apparent morphology of the genome changes during the cell cycle. It is thought that the tight packing of DNA prevents or suppresses gene expression, and that loosening or unfolding is essenual for transcription into RNA. The structural transition between the unfolded coil and folded compact states, or coil-globule transition [3], of giant DNA molecules should therefore be of great biological significance.

Polyamines, such as spermidine and spermine, are widely found in both prokaryotes and eukaryotes [4,5]. Since polyamines are multivalent cations under physiological conditions, they probably participate in many cellular processes through their binding to negatively charged DNA and RNA. Past studies have indicated that polyamines strong-

*Corresponding author. Tel.: +81-75-753-3812; fax: +81-75-753-3779.
E-mail address: yoshikaw@scphys.kyoto-u.ac.jp (K. Yoshikawa).

0301-4622/02/$ - see front matter
PII: S0301-4622(02)00110-2

ly affect many metabolic pathways, particularly those involving nucleic acids, through the processes of replication [6], transcription [7–9] and translation [10,11].

There have been many reports on the interaction between polyamines and DNA with regard to the binding equilibrium [12–14], kinetics [15,16], condensation/aggregation [15–27] and morphology of the binding products [18,20,22]. Gosule and Schellman [17] reported that the addition of polyamines with three or four positive charges leads to cooperative 'DNA condensation'. They concluded that the reaction was very rapid and that DNA remained in the B-form. Up to the mid-1990s, studies on 'DNA condensation' [15–30] have dealt with the behavior of multiple DNA molecules, or at least with phenomena involving both single and multiple molecules. The general consensus has been that 'DNA condensation' is a steep but continuous process. For example, through the careful measurement of 'DNA condensation' with light scattering, Widom and Baldwin [28] concluded that (1) the transition is not a two-state reaction and (2) the transition for monomolecular condensation is diffuse. Unfortunately, these conclusions were based on measurements for an ensemble of DNA molecules. Recently, with single-molecule observation by fluorescence microscopy, it has been clarified that individual DNAs undergo a large discrete folding transition, or an all-or-none transition, between the unfolded and folded states upon the addition of various kinds of condensing agents, such as polyamines [31], multivalent metal cations [32,33], hydrophilic polymers [34], cationic polymers [35], cationic surfactants [36] and non-ionic surfactants [37]. Regarding the folding transition of single DNA molecules induced by polyamines [31,38], it has been shown that the critical concentrations of diamine (1,3-diaminopropane), triamine (spermidine) and tetraamine (spermine) for inducing the transition decrease in the order of valency.

Some recent papers [23,25–27] concerning 'DNA condensation' published after the mid-1990s have addressed this new insight regarding the discrete nature of this transition. The effect of temperature on the folding/unfolding transition at a fixed concentration of spermidine has also been examined using single-DNA observations [39]. It has been found that DNA chains fold into a compact state with an increase in temperature. Based on theoretical considerations [31,32] regarding statistical thermodynamics in the folding/unfolding transition induced by polycations and the measured electrophoretic mobility of DNAs [40], it has been concluded that ion exchange between monovalent and multivalent ions plays an important role in stabilizing the folded compacted state in single DNA molecules.

A few reports have described the effect of pH in the protein-DNA interactions [41] and the DNA condensation induced by polyamines [20,21]. Lohman et al. [41] reported that the binding constant for pentalysine-T7 DNA decreased with increasing pH. Allison et al. [20] obtained electron microscopic images of viral DNA in the presence of triamines (spermidine and its homologs), and found that both the size and shape change drastically at pH 10.2. On the other hand, Thomas and Bloomfield [21] reported that there was no substantial difference in spermidine-induced DNA condensation between pH 5.1 and 10.2. The observations of both Allison et al. and Thomas and Bloomfield indicated that pH had almost no effect on the 'DNA condensation' induced by polyamines at physiological pH. However, the methods used in these studies, such as light scattering, primarily provide information regarding the physico-chemical characteristics of the ensemble of chains, without a clear discrimination between single-chain compaction and multiple-chain condensation. Furthermore, in measurements with electron microscopy, it is almost impossible to accurately examine the effect of a change in pH. In the present paper, we show that an all-or-none structural change is generated around the physiological pH range in the presence of the tetramine spermine.

2. Experimental section

Bacteriophage T4 DNA (166 kbp) was purchased from Sigma–Aldrich Japan (Tokyo, Japan). The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Spermine tetrahy-

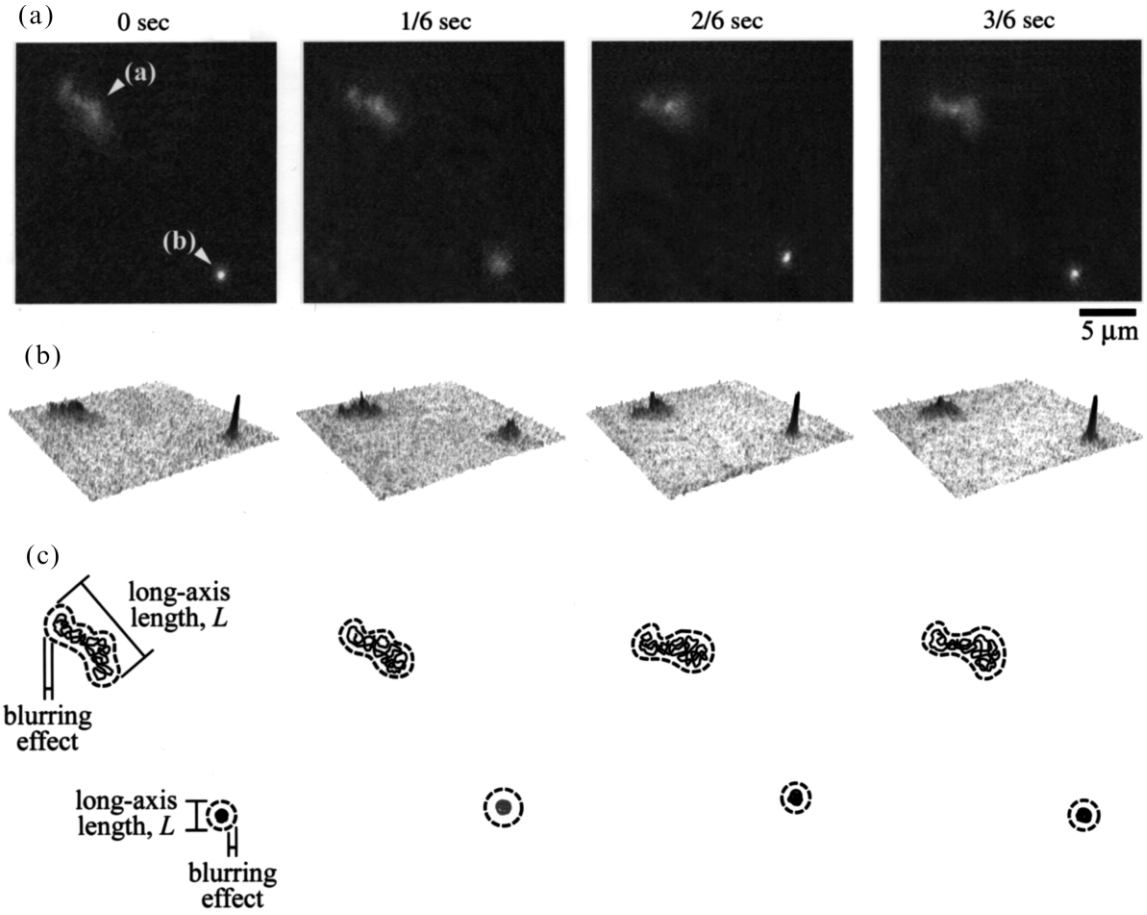

Fig. 1. (a) Fluorescence image of the thermal fluctuation of single T4 DNA molecules in Tris–HCl buffer solution (pH 7.0) in the presence of 6.0 μM spermine: (a) elongated unfolded state; (b) compact folded state. The time interval is 1/6 s. (b) Fluorescent light-intensity distributions for the image in (a). (c) Schematic representation of the actual conformation of single double-stranded DNA molecules. The apparent sizes in the fluorescence microscopy images are larger than the actual size due to the blurring effect of approximately 0.3 μm. The shallowing of the fluorescence image of the folded DNA in the second image is due to an out-of-focus effect, induced by translational Brownian motion.

drochloride (SPM) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

T4 DNA was dissolved in 25 mM Tris–HCl buffer solution of pH 5.8–8.9 with 0.1 μM DAPI, and was adjusted to a final concentration of 0.1 μM in base units. It has been previously confirmed that the persistence and contour lengths of DNA remain essentially constant at such a low concentration of DAPI [42].

Fluorescence microscopic measurements were performed as follows. The samples were illuminated with 365-nm UV light, and fluorescence images of DNA molecules were observed using a Carl Zeiss Axiovert 135 TV microscope equipped with a 100× oil-immersed lens. Images were recorded on videotape at one frame per 1/30 s through a high-sensitivity SIT TV camera and an image processor Argus 10 (Hamamatsu Photonics). Observations were carried out at 20 °C. To characterize the size of DNA, we measured the long-axis length, L, which was defined as the longest distance in the outline of DNA images (see Fig. 1). Due to the blurring effect of fluorescence light, the apparent size L in a DNA image

is larger than that of actual DNA, approximately 0.6 μm [34]. To minimize the adsorption of DNA molecules onto the glass surface, special care was taken to clean the glass microscope slides and cover-slips before the observation: they were baked in an electric oven at 500 °C for 1 h, soaked in hydrogen peroxide and ethanol for more than half a day, respectively, and finally washed with MilliQ water.

3. Results

Fig. 1 shows an example of the fluorescence images of thermally fluctuating single T4 DNA molecules in Tris–HCl buffer solution (pH 7.0) in the presence of 6.0 μM spermine, where the unfolded coil and folded compact conformations coexist in the solution. In the actual measurement, discrimination between the unfolded and folded states is easy, by using successive video frames based on the observation of intramolecular thermal fluctuation. The manner of Brownian motion of DNA molecules is markedly different between the elongated and folded states. While the unfolded DNA molecule exhibits relatively slow translational fluctuation, it shows marked intrachain thermal motion. In contrast, for the folded DNA molecule, significant translational motion is observed without apparent intrachain fluctuation. To obtain qualitative evaluation of the translational Brownian motion of individual DNA molecules, we measured the hydrodynamic radius by analyzing the translational diffusion constant D of individual molecules [33,38,43]. The value of D can be obtained from the mean-square displacement of the center of mass for individual DNA molecules. We found that the hydrodynamic radius in the folded state (0.085 ± 0.013 μm) is approximately $1/10$ that in the unfolded state (0.79 ± 0.18 μm).

Fig. 2 shows the dependence of the long-axis length, L, of T4 DNA molecules on the concentration of spermine at pH 7.0. In the absence and even in the presence of spermine up to 5 μM, individual DNA molecules assume the elongated state. The rather broad distribution is due to the intrinsic nature of the elongated coil state, i.e. the coil state exhibits large thermal fluctuations. With an increase in the concentration of spermine, DNA

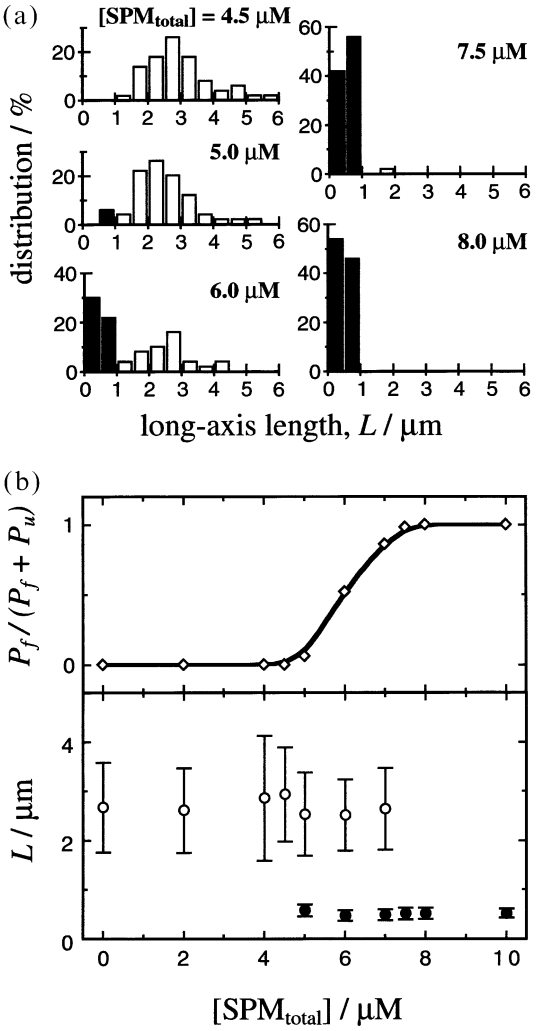

Fig. 2. (a) Histogram of the long-axis length, L, of T4 DNA molecules at various concentrations of spermine. The area of each histogram has been normalized to be equal. The open and closed columns correspond to the elongated unfolded and compact folded states, respectively. (b) Total concentration of spermine vs. the ratio of folded DNAs, P_f, to the total DNA molecules, $P_f + P_u$, (top), or long-axis length, L, of T4 DNA molecules (bottom). The open and closed circles show the mean value of L in the elongated and folded states, respectively. The vertical bars show the standard deviation. The transition is all-or-none at the level of individual DNA molecules. In this experiment, pH was fixed at 7.0, and at least 50 DNA molecules were analyzed for each concentration of spermine.

folds into the compact state. Between the two extreme states in which all of the DNAs show either an unfolded coil or folded compact conformation, there is a region where the unfolded and compact states coexist, as in Fig. 1. Fig. 2b shows the change in the long-axis length, L, and the ratio of folded DNAs, P_f, to total DNA molecules, $P_f + P_f$ as a function of the spermine concentration [SPM_{total}]. The concentration-dependence of the folding transition observed here is essentially the same as that reported previously [31].

Fig. 3 shows the dependence of the long-axis length, L, of T4 DNA molecules on pH in the presence of a fixed concentration of spermine (7.5 μM). In acidic pH, all of the DNA molecules exist in the compact folded state. With an increase in pH, compact DNA molecules unfold into the elongated conformation. At pH 8.9, all of the DNAs are completely unfolded. Thus, at a fixed concentration of spermine, individual DNA chains undergo large discrete changes in their conformation with a change in pH. Fig. 3b (top) shows the ratio of the concentration of tetra-ionic species, [SPM^{4+}], to the total concentration of spermine, [SPM_{total}], calculated from the pK_as of spermine, 7.96, 8.85, 10.02 and 10.80, at 25 °C [44]. Fig. 3b also shows the dependence of the long-axis length, L, and the ratio of folded DNAs, P_f, to total DNA molecules, $P_f + P_u$ Based on this figure, it is apparent that the change of [SPM^{4+}] in the solution is the main factor in the folding/unfolding transition induced by a change in pH.

The results of the two structural transitions, depending on the pH or [SPM_{total}], were compared with regard to the region of coexistence between the folded and unfolded states (Fig. 4a). [SPM^{4+}] was calculated from the dissociation constants (Fig. 3b, top). Here, it is to be noted that the apparent deviation on the curve of [SPM^{4+}]/[SPM_{total}] from that of $P_f/(P_f+P_u)$ as in Fig. 3b is referenced to a linear relationship, through the re-scaling of these parameters. For each data point in Fig. 4a, we measured more than 400 DNA molecules at a given pH and [SPM_{total}] all to gain more precise insight into the pH dependence. For the closed circles, [SPM^{4+}] was estimated by measuring the pH dependence at a fixed spermine concentration, as in Fig. 3. On the other hand, for

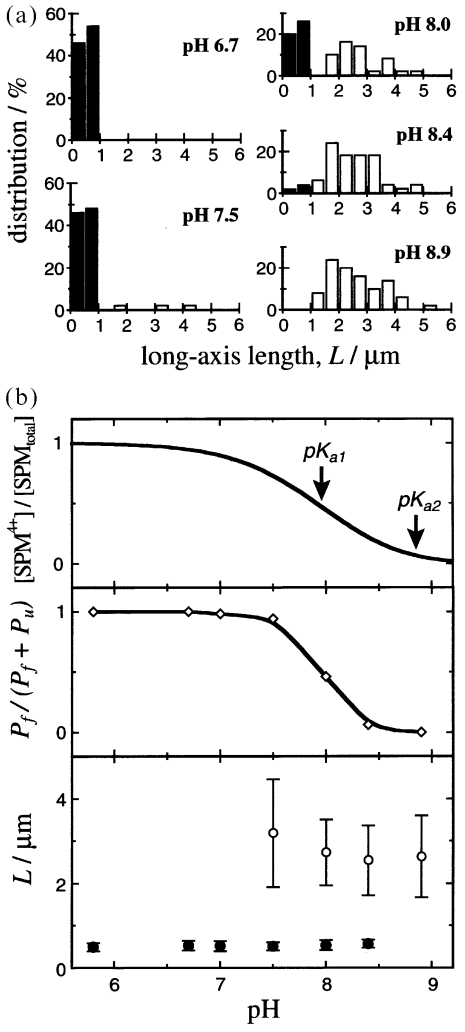

Fig. 3. (a) Histogram of the long-axis length, L, of T4 DNA molecules in solutions of various pH values with a fixed concentration of spermine (7.5 μM). The open and closed columns correspond to the elongated unfolded and compact folded states, respectively. (b) pH in the presence of a fixed concentration of spermine (7.5 μM) vs. the ratio of tetravalent spcrmine, SPM^{4+}, to the total concentration of spermine, SPM_{total}, (top), the ratio of folded DNAs, P_f, with respect to the total DNA molecules, $P_f + P_u$, (middle) or the long-axis length, L, of T4 DNA molecules (bottom). The open and closed circles show the mean value of L in the elongated and folded states, respectively. The vertical bars show the standard deviation. At least 50 DNA molecules were analyzed for each pH. The ratio of SpM^{4+} was calculated from the dissociation equilibrium and four acidity constants for spermine; pK_{a1} = 7.96, pK_{a2} = 8.85, pK_{a3} = 10.02 and pK_{a4} = 10.80 at 25 °C [44].

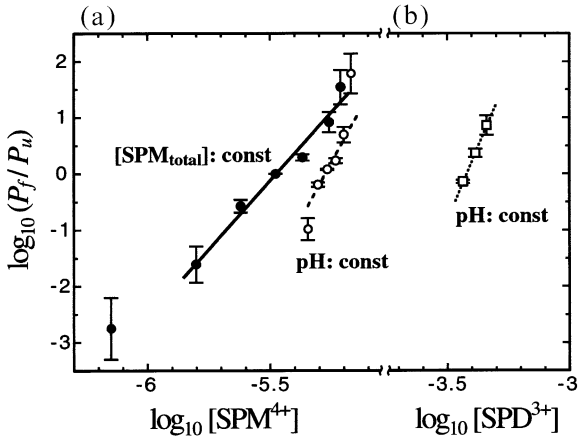

Fig. 4. Dependence of the ratio of the folded and unfolded states on the concentrations of (A) spermine [SPM^{4+}] and (B) spermidine [SPD^{3+}]. P_f and P_u, are the percentages of folded and unfolded DNAs. The closed and open circles show the pH dependence at a fixed spermine concentration (7.5 μM) and spermine dependence at a fixed pH (7.0), respectively. At least 400 DNA molecules were counted for each data point (closed and open circles). The open squares show spermidine dependence in pH 7.3 TE buffer solution [45]. The vertical bars indicate error bars.

the open circles, [SPM^{4+}] was estimated by measuring the dependence on the concentration of spermine at a fixed pH, as in Fig. 2. The slopes are 5 and 8 for the former (closed circles) and latter (open circles) cases, respectively. In Fig. 4b, the open squares are based on the dependence on the concentration of spermidine (SPD^{3+}). The slope, or the 'apparent' cooperativity, is 10 [45], being slightly larger than that in SPM^{4+}. It is to be noted that the slope, *n*, corresponds to the so-called Hill coefficient [46]. In the next section, we will discuss the 'apparent' cooperativity, in relation to the mechanism of the transition.

4. Discussion

The present results indicate that individual DNA molecules undergo an all-or-none transition from a compact state to an elongated state with an increase in pH from 6.7 to 8.9 in the presence of 7.5 μM spermine. Based on studies of the folding transition of DNA induced by multivalent cations using single DNA-molecule observations, the following properties have become clear [31,39,40,47]. (i) The transition between the unfolded and folded states on giant DNA above the size of several tens of kbp is largely discrete at the level of individual molecular chains, whereas the transition appears to be continuous for the ensemble of chains. (ii) In folded compact DNA, while the negative charge completely disappears overall, the folded product behaves similarly to a charged colloid. Due to this colloidal nature, around the transition region, folded compact DNA dissolves in solution without aggregation. With an increase in the concentration of condensing agents, in this case multivalent cations, folded DNA molecules tend to stick to each other and form an assembly of multiple chains. (iii) The folding transition is promoted by a decrease in salt concentration and by an increase in temperature. This fact indicates that ion exchange between monovalent and multivalent cations, i.e. an increase in the translational entropy of small ions, plays an important role in the mechanism of the folding transition. (iv) The critical concentrations for inducing the folding transition are roughly 1:1/10:1/100 for divalent (1,3-diaminopropane), trivalent (spermidine) and tetravalent (spermine) polyamines, respectively.

Based on the above-mentioned property of the folding transition induced by multivalent cations, we would like to extend our discussion of the mechanism of the folding/unfolding transition induced by a change in pH. The DNA unfolding transition with increasing pH is associated with a decrease in [SPM^{4+}]. In view of the above-mentioned point (iv) on the marked difference of the potency between trivalent and tetravalent species, [SPM^{4+}] is expected to be the main factor that influences the transition. Most spermine will be in the form SPM^{4+}, or SPM^{3+} at approximately pH 6–9 under the dissociation equilibria:

$$SPM^{4+} \overset{K_{a1}}{\rightleftarrows} SPM^{3+} + H^{+} \quad (1)$$

and

$$SPM^{3+} \overset{K_{a2}}{\rightleftarrows} SPM^{2+} + H^{+} \quad (2)$$

where K_{a1}, and K_{a2} are dissociation constants:

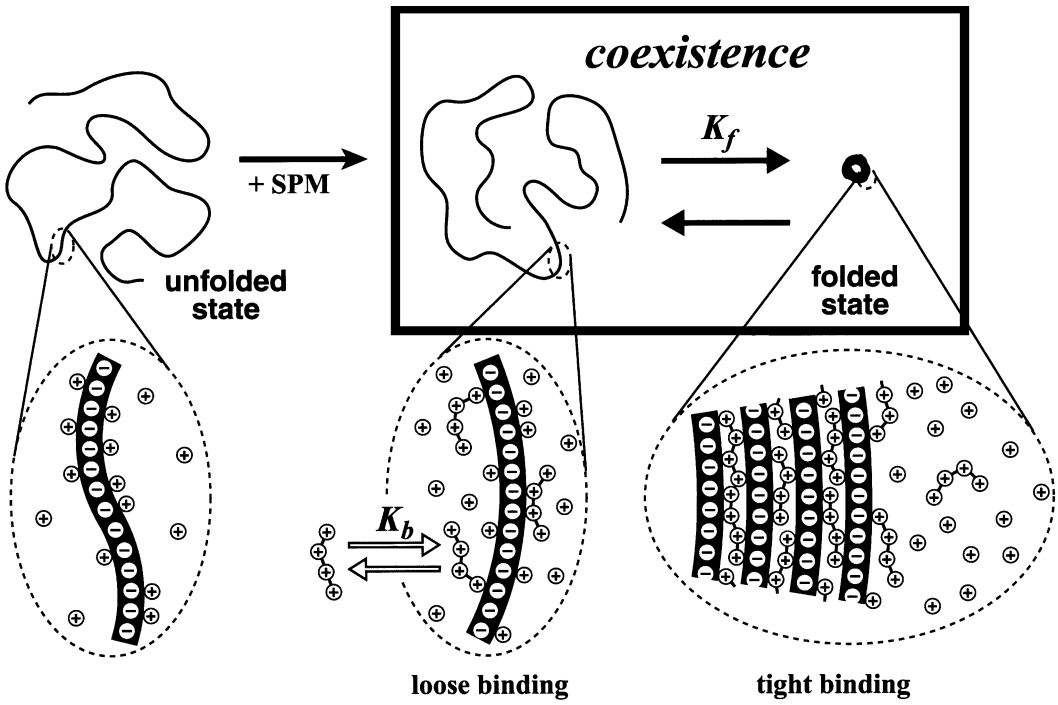

Fig. 5. Schematic representation of the folding transition of DNA molecules with the addition of spermine. K_b is the binding constant of spermine^{4+} to phosphate groups in an elongated DNA chain. K_f is the equilibrium constant between the elongated and compacted states of DNA, where the manner of binding of spermine changes dramatically.

$$K_{a1} = \frac{[\mathrm{SPM}^{3+}][\mathrm{H}^{+}]}{\mathrm{SPM}^{4+}} \qquad \mathrm{p}K_{a1} = 7.96 \qquad (3)$$

$$K_{a2} = \frac{[\mathrm{SPM}^{2+}][\mathrm{H}^{+}]}{\mathrm{SPM}^{3+}} \qquad \mathrm{p}K_{a2} = 8.85 \qquad (4)$$

Based on the present and previous results [31,39,40], the effect of spermine on DNA can be schematically depicted as in Fig. 5. When the spermine concentration is low, spermine will interact with the negative charge on DNA through a dynamic binding equilibrium, i.e. spermine loosely binds to the phosphate groups along double-helical DNA under large thermal fluctuations. Thus, the effective charge on DNA decreases gradually with an increase in the spermine concentration. According to the counter-ion condensation theory [29,30], the negative charge of DNA is neutralized by approximately 70% in normal aqueous solution in the absence of multivalent cations. The number of SPM^{4+} molecules (α) loosely bound to individual DNAs will increase with an increase in the bulk concentration of spermine, and thus the degree of the charge neutralization increases. It has been suggested that the negative charge is neutralized by up to 90% just before 'DNA condensation' [16,19,29,30]. A similar decrease in the negative charge on elongated DNA was confirmed in a recent study by Yamasaki et al. [40]. Based on single-chain observation with fluorescence microscopy, as in Figs. 2 and 3, the long-axis length, L, remains almost constant as long as DNA remains in the elongated state, regardless of the concentration of spermine. Thus, at the level of single DNA chains, approximately 10% of the negative charge on elongated-coil DNA is not neutralized in the region of coexistence; i.e. due to the remaining 10% of the negative charge, the DNAs assume an elongated conformation with essentially the same long-axis length, even in the presence of spermine. This situation is schematically represented in Fig.

5 by considering the loose binding of spermine. If the decrease in the negative charge, from 30 to 10% in the coil state, is due to such loose binding, the number of loosely bound spermine molecules would be almost enough to induce the complete charge neutralization seen with strong binding, as shown in Fig. 5 (bottom right). Regarding the binding equilibrium, Record et al. [48] reported a thermodynamic analysis that included the conformational change in the second-order structure, or helix-coil transition. However, their analysis cannot be applied to the folding transition of DNA, since they did not take into account the effect of the large discrete transition in the higher-order structure of DNA. Thus, in the present study, we performed a thermodynamic analysis that includes a large change in the conformation of DNA.

DNA chains in the coexistence region can be considered to be in a kind of equilibrium between the elongated and compact states:

$$[\mathrm{DNA}_u \cdot \mathrm{SPM}_\alpha] + \beta \mathrm{SPM} \overset{K_f}{\rightleftarrows} [\mathrm{DNA}_f \cdot \mathrm{SPM}_{\alpha+\beta}] \qquad (5)$$

where K_f is the equilibrium constant, α is the number of spermine molecules weakly bound to a single unfolded DNA chain and $\alpha+\beta$ is the number of spermine molecules tightly bound to a folded DNA. DNA_u, and DNA_f indicate the unfolded and folded states, respectively. β is the apparent number of spermine molecules that attach to DNA chains while they are undergoing the folding transition. From the equilibrium in Eq. (5), it is clear that β corresponds to the cooperativity parameter n, the Hill coefficient, as reflected in the slopes in Fig. 4, in spite of the large difference in the physico-chemical mechanism.

Recently, a thermodynamic study of single T4 DNA molecules was performed using changes in temperature [39]. In the presence of a fixed amount of spermidine, DNA molecules exhibit a transition from a compact state to an unfolded state with increasing temperature, i.e. the disperse state is stabilized at higher temperatures. This indicates that ion exchange between trivalent spermidine and monovalent counter ions (Na^+) plays an important role in the mechanism of transition. In this experiment, the apparent change in entropy for the spermidine-induced folding transition was evaluated to be $+32\pm8\ k_B$ per T4 DNA molecule.

Note that the translational entropy for individual ions is roughly on the order of the apparent cooperative parameter β. It is somewhat surprising that the change in entropy in this transition is of the order of 10 k_B, when we consider the size of T4 DNA molecules. T4 DNA has approximately 3×10^5 phosphate groups. Even when 90% of the negative charge is masked by the condensation of counter ions, as in the coil state in the region of coexistence, as many as 3×10^4 negative charges still survive.

A similar situation would hold for the folding transition induced by spermine. If complete charge neutralization occurred with spermine supplied from the bulk solution, the change in the translational entropy of counter ions should also be of the order of at least 10^3–10^4. This problem can be solved by considering the change in the manner of binding of spermine in the folding transition. Based on this consideration, the actual number of additional spermine molecules needed to bind to DNA, corresponding to β, is expected to be on the order of ten per T4 DNA molecule.

There have been several reports on the change in the higher-order structures of DNA and chromatin with a change in pH. Gerson [49] reported a change in pH in Physarum from 5.9 in interphase to 6.8 during mitosis, and suggested that the rise in pH in mitosis might stimulate DNA synthesis and division. Although the degree of protonation of spermine is not sensitive to this range of pH, we can expect a change in the dissociation of various ion species in the intracellular medium. Thus, changes in the dissociation of these species may cause a change in the conformation of DNA, and this change may induce other biological changes. Guo and Cole [50] also discussed the possible role of a change in pH based on the in vitro observation of chromatin aggregation dependent on the change in pH within the physiological range. They proposed that an increase in pH would stimulate DNA synthesis through the decondensation of chromatin in a living cellular system. The pH-dcpendent change in the stability of chromatin fragments has also been reported [51]. Changes in intracellular pH have also attracted much interest

with regard to apoptosis (programmed cell death), in which chromatin undergoes significant morphological changes [52–54]. It is generally considered that binding of transcription factors to DNA causes ON/OFF switching on target genes. The number of members in all transcription factor families increases in the order of yeast, nematode, fruit fly and human [55]. The diversity of cell types in these organisms also increases in this order [56]. This makes sense, given that maintaining the differentiated states of increasingly diverse cell types requires the presence of increasingly more molecular switching [57]. It remains unclear how living cells self-regulate such a very complicated network with a large number of 'regulatory' factors [58]. Therefore, the presence of some other mechanisms for the self-regulation of gene expression should be considered in addition to the complicated key-lock interactions of regulatory proteins. Bork and Copley [59] suggested that the cellular environment and genome packing might be regulatory factors. They speculated that the environment that the gene shared with other species may offer insight into function and regulation beyond the level of individual genes, and that the three-dimensional packing of the genome in a cell's nucleus is likely to influence gene regulation. Considering the ON/OFF switching of gene expression in living cells, it seems reasonable to expect that transcription should be affected by the transition in the higher-order structure of genomic DNA with changes in the concentration or distribution of abundant, non-specific materials [47,60] such as polyamines, ATP [45] and RNA [43].

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